Biochemical Pharmacology, Vol. 27, pp. 317–321. Pergamon Press, 1978. Printed in Great Britain.

SPIPERONE: A LIGAND OF CHOICE FOR NEUROLEPTIC RECEPTORS

2. REGIONAL DISTRIBUTION AND *IN VIVO* DISPLACEMENT OF NEUROLEPTIC DRUGS

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(*Received* 4 *May* 1977; *accepted* 8 *July* 1977)

Abstract—Specific *in vitro* as well as *in vivo* binding of neuroleptic drugs was demonstrated by studying the regional distribution in rat brain. [^{3}H]spiperone and [^{3}H]pimozide were found to be specifically taken up in the dopaminergic areas of the brain, a fact which correlates with the distribution of neuroleptic receptors when measured under *in vitro* conditions. Larger doses of unlabeled neuroleptics only displaced the labeled neuroleptic *in vivo* in the dopaminergic areas (striatum, nucleus accumbens, tuberculum olfactorium and frontal cortex) but not in the cerebellum. Dopamine agonists were found to partly displace the labeled spiperone in the striatum, thus providing further evidence about the dopaminergic nature of the neuroleptic receptor. Nevertheless, some *in vivo* experiments suggest that the neuroleptic receptor is not the same in all the dopaminergic areas. It is concluded that spiperone is a ligand of choice for *in vivo* studies of neuroleptic receptors.

Since most ligands, agonist or antagonist, are able to bind to membrane structures *in vitro* as well as *in vivo*, various criteria must be fulfilled to identify specific receptor binding. For instance, binding of ligands *in vitro* should be saturable and stereospecific and the affinity of a large number of compounds for the binding sites should parallel their pharmacological activity *in vivo* (cf. previous paper).

The regional distribution can provide additional evidence to demonstrate specific bindings, especially concerning *in vivo* binding experiments. Unlike certain peripheral tissues, the brain can be dissected into various areas containing a given enzyme or a neurotransmitter.

For many years, neuroleptics were thought to act by blocking dopamine receptors. The recent finding that brain receptors can stereospecifically bind labeled haloperidol has provided more direct evidence to support this hypothesis [1, 2]. One of the main features of this binding assay is that it can almost exclusively be performed using homogenates obtained from brain dopaminergic regions [1–4].

The purpose of the present paper is to report the regional distribution of *in vitro* as well as *in vivo* [^{3}H]spiperone binding in rat brain. Moreover displacement experiments of [^{3}H]spiperone and [^{3}H]pimozide in different brain regions will be presented in order to characterize the *in vivo* binding sites of neuroleptic drugs. A preliminary account of this work has been published [5].

MATERIALS AND METHODS

Male Wistar rats (approximatively 250 g) were i.v. injected with the labeled and unlabeled drug. At different time intervals after injection, the animals were killed by decapitation and the brains were removed. Most of the brain areas were dissected according to Glowinski and Iversen [6] whereas the nucleus accumbens and the substantia nigra were sucked up through a Pasteur micropipette (1.5 mm inner diameter) according to the coordinates of De Groot [7]. Brain samples were combusted in a tissue sample oxidizer and the radioactivity was counted in a liquid scintillation spectrometer.

In control experiments, the radioactivity was extracted from tissue samples by a mixture heptane–isoamyl alcohol (8.5:1.5). After evaporation, the sample was analyzed by thin layer chromatography (solvent–benzene–ethanol, NH_4OH 90:10:1).

The *in vitro* binding using [^{3}H]spiperone was assayed in a total particulate fraction as described in the previous paper [8].

Homovanillic acid (HVA) determination. Tissue samples were homogenized in 6 vol. of 0.7% perchloric acid. After centrifugation, 4 ml of the supernatant was extracted with 6 ml of freshly distilled ethylacetate. After shaking and centrifugation, 5 ml of the organic phase was removed and extracted with 2.6 ml of 0.5 M. Tris–HCl buffer pH 8.5. HVA was measured according to Anden *et al.* [9] using internal standards.

Drug. [^{3}H]spiperone (sp. act. 9 Ci/m-mole), [^{3}H]haloperidol (sp. act. 8.5 Ci/m-mole) and [^{3}H]pimozide (sp. act. 13 Ci/m-mole) were obtained from the Radiochemical Department of Janssen Pharmaceutica. The labeled and unlabeled drugs were dissolved in saline to which two drops of lactic acid were added. 2-(*N*,*N*-dipropyl) amino 5,6-dihydroxytetralin (AMT) was donated by Dr. J. Cannon. Apomorphine was supplied by E. Merck, Darmstadt.

RESULTS

Regional distribution of in vitro [^{3}H]*spiperone binding in rat brain.* Stereospecific [^{3}H]spiperone binding was assayed in homogenates from various rat brain

Table 1. Regional distribution of radioactivity in rat brain 2 hr after i.v. injection of labeled neuroleptic drugs (*in vivo* binding) and of spiperone binding measured under *in vitro* conditions

Brain region	*In vivo* binding pg mg^{-1}				*In vitro* binding pmoles g^{-1}
	[^{3}H]Spiperone		[^{3}H]Haloperidol	[^{3}H]Pimozide	
	0.005 mg kg^{-1}	0.63 mg kg^{-1}	0.005 mg kg^{-1}	0.02 mg kg^{-1}	[^{3}H]Spiperone
Nucleus accumbens	2.79 ± 0.38	70.6 ± 6.3	9.7 ± 0.5	6.4 ± 1.3	26.8 ± 2.0
Striatum	2.50 ± 0.15	61.7 ± 1.9	11.8 ± 0.4	7.6 ± 0.5	40.9 ± 0.9
Substantia nigra	1.71 ± 0.3	49.6 ± 5.7	9.9 ± 1.1	4.1 ± 0.2	27.1 ± 0.6
Tuberculum olfactorium	1.59 ± 0.08	56.1 ± 3.9	11.0 ± 0.2	5.1 ± 0.4	24.4 ± 1.6
Frontal cortex	1.02 ± 0.05	51.1 ± 4.3	12.9 ± 0.2	4.3 ± 0.2	21.9 ± 1.3
Parietal cortex	0.74 ± 0.06	42.1 ± 2.9	14.3 ± 0.3	4.1 ± 0.2	15.3 ± 2.1
Occipital cortex		40.6 ± 1.8	12.3 ± 0.3	4.0 ± 0.2	10.3 ± 1.1
Hypothalamus		34.9 ± 3.5	10.3 ± 0.3		7.6 ± 1.2
Hippocampus		36.5 ± 2.6	9.0 ± 0.2		
Medulla oblongata	0.77 ± 0.06	33.5 ± 1.3	13.7 ± 0.1	3.4 ± 0.2	4.6 ± 0.9
Thalamus		31.9 ± 1.4	11.1 ± 0.3		5.8 ± 1.0
Olfactory bulb		40.9 ± 3.8	8.3 ± 0.2		6.4 ± 0.7
Spinal cord		50.4 ± 4.2	11.0 ± 0.3		2.6 ± 0.7
Cerebellum	0.29 ± 0.02	31.7 ± 1.3	10.6 ± 0.2	2.7 ± 0.3	3.3 ± 0.7

*Results are the mean of 4–6 determinations (± S. E. M.).

areas. Table 1 shows clearly that spiperone binding sites are mainly located in the dopaminergic areas (striatum, substantia nigra, nucleus accumbens, olfactory tubercle and frontal cortex). The striatum appears to contain the largest number of receptor sites followed by the substantia nigra, the nucleus accumbens and the olfactory tubercle.

Interestingly, a relatively large amount of spiperone binding was detected in the frontal cortex in contrast with the parietal and even more the occipital cortex. In the non-dopaminergic areas, especially in the cerebellum and spinal cord, there are very few spiperone binding sites.

Regional distribution of labeled neuroleptic drugs in vivo. Different doses of three labeled neuroleptic drugs, two belonging to the butyrophenone series and one (pimozide) to the diphenylbutylpiperidine series, were injected intravenously into rats. Two hours later the total radioactivity, was determined in various brain regions. Table 1 shows the distribution of these neuroleptic drugs in rat brain. When [^{3}H]spiperone was used at a very low dose (5 μg/kg), it was preferentially taken up in the dopaminergic areas. Indeed the retention capacity in the striatum and the nucleus accumbens was found to be about 10 times that of the cerebellum. Other dopaminergic areas, such as the substantia nigra, the olfactory tubercle and even the frontal cortex also revealed a relatively high amount of radioactivity. Figure 1 shows that this regional distribution, thus performed under *in vivo* conditions, nicely correlates with that of the *in vitro* binding.

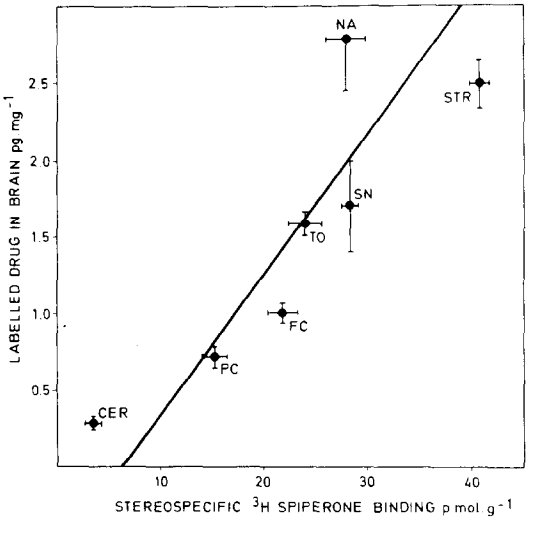

Fig. 1. Rat brain regions: correlation between the radioactivity measured in rat brain after i.v. injection of [^{3}H]spiperone and the *in vitro* stereospecific [^{3}H]spiperone binding assay. The spearman rank correlation coefficient $r_s = 0.85$. NA, nucleus accumbens; STR, striatum; SN, substantia nigra; TO, tuberculum olfactorium; FC, frontal cortex; PC, parietal cortex; CER, cerebellum.

However, when using a higher dose of labelled spiperone (0.63 mg kg^{-1}), the difference in the retention capacity between the dopaminergic and non-dopaminergic areas, though still being detectable, was much less pronounced. Similar results were obtained with a low dose of [^{3}H]pimozide. Here, however, there was no difference between the three cortices and the difference in the retention capacity between the nucleus accumbens and the cerebellum was smaller than when using a low dose of [^{3}H]spiperone. Surprisingly, labeled haloperidol although injected at very low dose (0.005 mg kg^{-1}) did not display a regional distribution with a higher retention capacity in the brain dopaminergic areas. Table 1 shows that after injection of labeled haloperidol, the radioactivity was more or less evenly distributed. Throughout this work, the radioactivity was always considered as unchanged drug. Chromatographic studies using TLC have shown that 2 hr after i.v. injection, 90 per cent of [^{3}H]spiperone and [^{3}H]pimozide were found in the rat brain as unchanged drug.

Displacement of labeled neuroleptic drugs in vivo. These experiments were carried out as follows: 1 hr after injection of labeled spiperone or pimozide, rats were given a much higher dose of the unlabeled drug. The radioactivity was measured at various time intervals in different brain areas of animals receiving either only the labeled drug or successively the labeled and unlabeled drug.

Figure 2 shows that after injection of [^{3}H]spiperone, a large amount of radioactive material was displaced in the brain of rats receiving a larger dose of unlabeled spiperone. However, the displacement was only detectable in dopaminergic regions, such as the striatum, the olfactory tubercle and the frontal

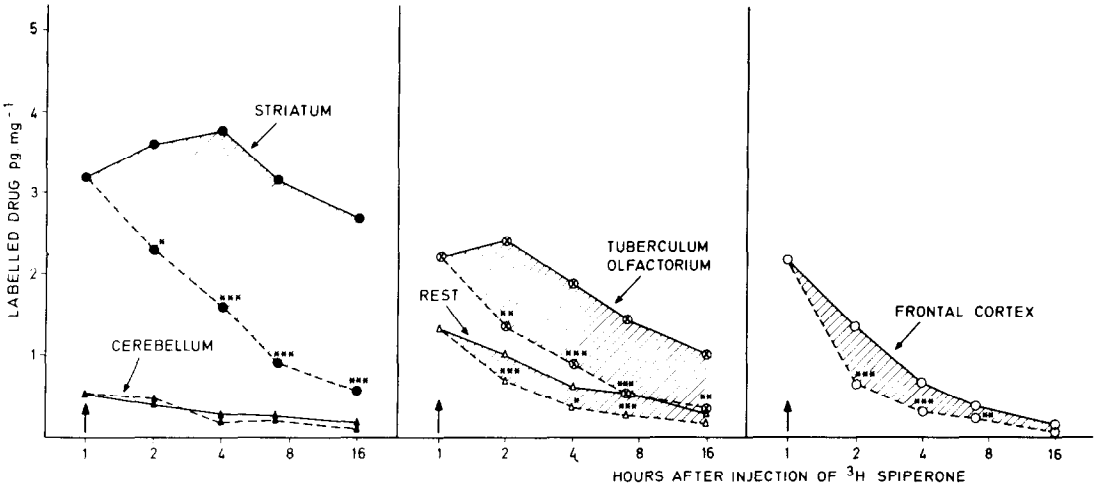

Fig. 2. *In vivo* displacement of [^{3}H]spiperone (0.005 mg kg^{-1} i.v.) in various rat brain regions. In the first group (——) rats received only labeled spiperone while in the second group (-----) 0.63 mg kg^{-1} (↑) unlabeled spiperone was injected i.v. 1 hr after [^{3}H]spiperone. The hatched part represents displaceable [^{3}H]spiperone. Each point is the mean of 4 determinations. Significant differences from control values are indicated by *P < 0.05 **P < 0.01 ***P < 0.001 (P: Student's *t*-test).

cortex but not in the cerebellum. In the rest of the brain which consists of the whole brain minus the three regions already quoted and the nucleus accumbens, the displacement was only modest.

It is noteworthy that the decline of the radioactivity was found to occur more rapidly in the frontal cortex than in the striatum.

Figure 3 shows a similar experiment but using [^{3}H]pimozide. Here again, a displacement of radioactive material was detected in the striatum, the nucleus accumbens and the olfactory tubercle but not in the cerebellum and the rest of the brain. When compared to spiperone, the distribution pattern is somewhat different; this point will be discussed later. Finally, in order to determine the nature of this *in vivo* binding, preliminary experiments were undertaken using dopamine agonists. For it, apomorphine and AMT were injected 1 hr after labeled spiperone. Figure 4A shows that apomorphine and AMT were able to displace partly the radioactivity in the striatum but not in the cerebellum. These results were compared to the effect of apomorphine on the HVA elevation induced by haloperidol treatment. As shown in Fig. 4B, apomorphine was found to produce a dose-related decrease of the HVA increase elicited by haloperidol in the rat striatum.

DISCUSSION

The regional distribution in rat brain of neuroleptic receptors using the [^{3}H]spiperone *in vitro* binding assay was found to differ somewhat from that previously found with [^{3}H]haloperidol [4, 10]. Indeed the present results provide a more clearcut differentia-

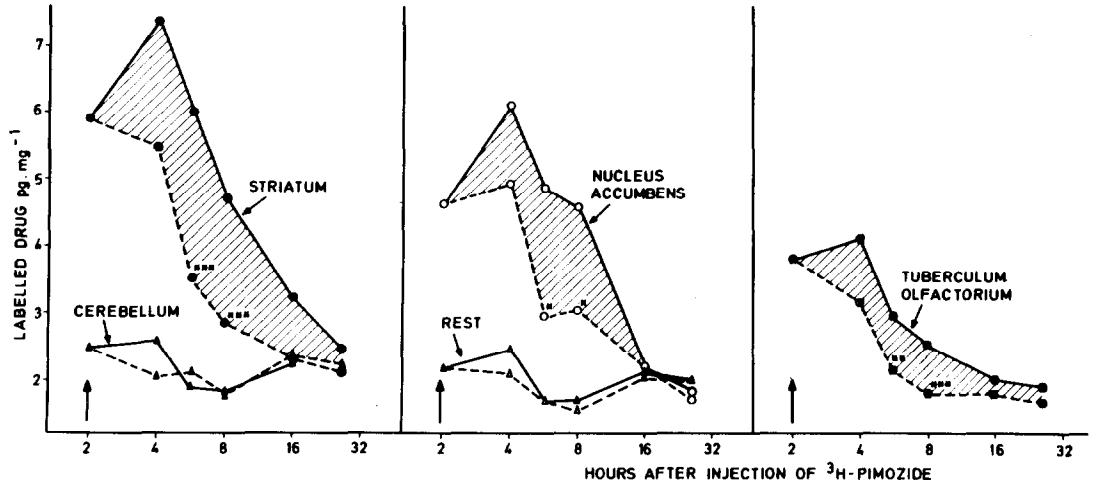

Fig. 3. *In vitro* displacement of [^{3}H]pimozide (0.02 mg kg^{-1} i.v.) in various rat brain regions. In the first group (——) rats received only labeled pimozide while in the second group (-----) 0.4 mg kg^{-1} (↑) unlabeled pimozide was injected i.v. 2 hr after [^{3}H]pimozide. See Fig. 2 legend for explanation of symbols.

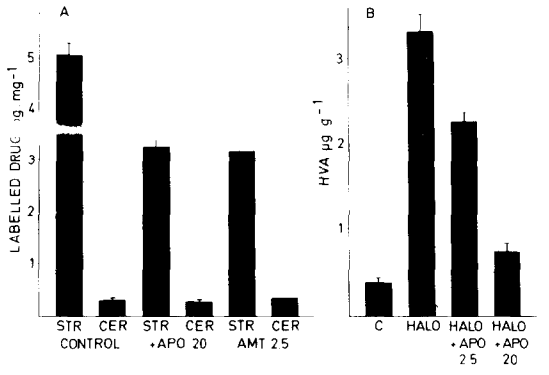

Fig. 4. (A) *In vivo* displacement of [^{3}H]spiperone (0.005 mg kg^{-1} i.v.) by dopamine agonists. Apomorphine (APO) 20 mg kg^{-1} and 2-(*N*,*N*-dipropyl) amino 5,6-dihydroxytetralin (AMT) 2.5 mg kg^{-1} were given intravenously to rats 1 hr after [^{3}H]spiperone. Rat brains were removed 3 hr later and the radioactivity was measured in the striatum (STR) and the cerebellum (CER). Each column except AMP ($n = 2$) represents the mean of 4 determinations (± S. E. M.). (B) Apomorphine antagonism of haloperidol-induced HVA increase in rat striatum. Haloperidol (Halo) 0.63 mg kg^{-1} was injected subcutaneously to rats. Fifteen min later apomorphine (APO; 2.5 and 20 mg kg^{-1}) was given intravenously and the brains were removed 1 hr later (mean of six rats ± S. E. M.).

tion in receptor sites between dopaminergic and non-dopaminergic areas. For instance, the spiperone binding assay has allowed us to clearly identify neuroleptic receptors in the frontal cortex, a fact which appears quite compatible with the occurrence of dopaminergic neurones in this part of the brain [11]. Moreover the difference in binding sites between the three cortex regions (frontal–parietal–occipital) parallels the different dopamine content observed in these three areas [12]. However, such a parallelism is not the rule in all the brain regions. The olfactory tubercle, for instance, contains much more DA than the frontal cortex or the substantia nigra [13] whereas their number of binding sites was found to be rather similar. This gives more support to the idea that the neuroleptic receptor when using spiperone is relatively heterogenous thus not only of a dopaminergic type but also of a serotonergic one, especially in the frontal cortex (in preparation).

From the present results, one may conclude that spiperone seems to be a much more appropriate ligand, perhaps the ligand of choice, for revealing the presence of neuroleptic receptors, especially in brain regions where they are only scarce in number. This is most probably due to the particular kinetic properties of the drug which have already been emphasized in the first paper [8]. Spiperone was found to have a very high affinity (10^9 M^{-1}) for the specific binding sites but a lower aspecific binding and a much slower dissociation rate than haloperidol.

These reasons, in particular the latter two, enabled us to show a specific regional distribution of [^{3}H]spiperone under *in vivo* conditions [5]. This was found to correlate with the regional distribution when measured under *in vitro* conditions. Therefore one may conclude that the retention capacity to accumulate spiperone in the dopaminergic areas is mainly due to the binding of the drug to specific neuroleptic receptors in these brain regions. Why is this not the case when using haloperidol? In fact, for reasons inverse to those which make spiperone more appropriate, although the affinity or haloperidol for the specific binding sites is still high, but 10 times lower than that of spiperone. In addition to this, haloperidol has a higher tendency towards aspecific adsorption and, perhaps the main reason, it has a very fast dissociation rate. Hence, our *in vitro* studies provide an explanation to the fact that haloperidol is not an adequate ligand for such *in vivo* studies, thus confirming previous observations [14, 15].

That aspecific adsorption is a limiting factor is further substantiated by the fact that the use of a higher dose of spiperone (0.63 mg kg^{-1}) was found to change dramatically the regional distribution, the difference between the striatum and the cerebellum becoming less pronounced.

In fact, when using higher doses, the aspecific binding keeps increasing and becomes so high that it masks the specific one which is very rapidly saturated. We are faced here with an identical situation concerning the receptor binding assays *in vitro*; a specific or stereospecific binding is only detectable if the concentration of the ligand is sufficiently low, about 10^{-9} M. At higher ligand concentrations, 10^{-7} M for instance, the aspecific binding, because it is not saturable, becomes predominant, thus masking the specific one. Therefore, it is of great importance to use extremely low doses of drug in order to evaluate *in vivo* specific binding. Moreover, as already mentioned, binding properties such as high affinity and slow dissociation rate are also required.

Pimozide also seems to be an appropriate ligand for *in vivo* studies although its aspecific binding under *in vitro* conditions was found to be higher than for spiperone and haloperidol (unpublished results). This probably explains the higher accumulation of this neuroleptic in the cerebellum when compared to spiperone and the lack of its retention in the frontal cortex. The displacement experiments provide further evidence for the specificity of the *in vivo* binding of neuroleptic drugs. Unlabeled drugs at high doses were able to displace the labeled drug bound in the dopaminergic areas (striatum, substantial nigra, nucleus accumbens, olfactory tubercle and frontal cortex) but not in the cerebellum. The lack of displacement in the latter area is interpreted as being due to aspecific bindings.

Surprisingly, the time-course of the [^{3}H]spiperone concentration was not identical in all the regions. The maximal uptake in the striatum was reached 4 hr after injection of the labeled drug whereas in the frontal cortex, the radioactivity content was already considerably decreased by that time. This feature cannot be attributed to a different amount of receptors in the dopaminergic areas but is most probably due to a difference in the nature of binding sites (in preparation). The fact that labeled spiperone remained bound for a longer period of time in the striatum than in the frontal cortex might suggest that the rate of dissociation, and thus the nature of the receptor sites in both areas, is not similar. Recent *in vitro* experiments have confirmed such a hypothesis (in preparation).

The spiperone *in vivo* binding in the striatum seems, at least, partly to occur on dopamine receptors since *in vivo*, apomorphine and AMT were able to displace labeled spiperone. It might be argued that the dose of apomorphine was relatively high but one has to recall that the affinity or apomorphine for the neuroleptic receptors is very low, about 100 times lower than that of spiperone. This displacement was found to correlate with the decrease of the homovanillic acid increase elicited by apomorphine when haloperidol was previously injected. This effect of apomorphine on haloperidol-induced homovanillic acid increase demonstrated the competitive nature of these drugs for the dopaminergic receptors [16].

Therefore the present results indicate that the spiperone binding in rat brain mainly occurs at the level of the dopamine receptors, thus confirming the results obtained under *in vitro* conditions [8]. However, in brain regions like the frontal cortex other kinds of receptor should also be involved. Spiperone was found to be the neuroleptic of choice for such *in vivo* studies.

Acknowledgements—Part of this work was supported by a grant from I.W.O.N.L. We thank M. Verwimp for her skilfull technical assistance and H. Van Hove for help in preparing the manuscript.

REFERENCES

1. I. Creese, D. R. Burt and S. H. Snyder, *Life Sci.* **17**, 993 (1975).
2. P. Seeman, M. Chau-Wong, J. Tedesco and K. Wong, *Proc. natn. Acad. Sci. U.S.A.* **72**, 4376 (1975).
3. P. Seeman, T. Lee, M. Chau-Wong, J. Tedesco and K. Wong, *Proc. natn. Acad. Sci. U.S.A.* **73**, 4354 (1976).
4. J. Leysen and P. Laduron, *Life Sci.* **20**, 281 (1977).
5. P. Laduron and J. Leysen, *Biochem. Pharmac.* **26**, 1003 (1977).
6. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 665 (1966).
7. J. De Groot, *Verh. K. Ned. Akad. Wet.* **52**, 14 (1959).
8. J. Leysen, W. Gommeren and P. Laduron, *Biochem. Pharmac.* (submitted for publication).
9. N. E. Anden, E. E. Roos and B. Werdinius, *Life Sci.* **7**, 448 (1963).
10. D. R. Burt, I. Creese and S. H. Snyder, *Molec. Pharmac.* **12**, 800 (1976).
11. A. M. Thierry, G. Blanc, A. Sohel, L. Stinus and J. Glowinski, *Science, N.Y.* **182**, 499 (1973).
12. W. Kerhr, M. Lindqvist and A. Carlsson, *J. Neural Transm.* **38**, 173 (1976).
13. D. H. G. Versteeg, J. Van der Gugten, W. de Jong and M. Palkovits, *Brain Res.* **113**, 563 (1976).
14. P. A. J. Janssen, W. Soudijn, I. van Wijgaarden and A. Dresse, *Arzneimittel-Forsch.* **18**, 282 (1968).
15. R. J. Naylor and J. E. Olley, *Br. J. Pharmac.* **36**, 208 (1969).
16. R. A. Lahti, B. McAllister and J. Wozniak, *Life Sci.* **11**, 605 (1972).